Technical Bulletin: Compatibility of the BAX® System and Environmental Sponges using a Wide Spectrum Neutralizer for the Detection of *Salmonella* species

The compatibility of the BAX® System and the 3M™ environmental scrub sampler with wide spectrum neutralizer was evaluated to detect *Salmonella* species from stainless steel surfaces. Low and high levels of *Salmonella* Typhimurium and a non-target competitor strain were co-inoculated in a 1:10 ratio onto unpaired sterile test areas. The inoculum was desiccated for 24 hours and then collected by swabbing. Half of the sponges were enriched in BPW according to the BAX® System method, and the second half were enriched in LB according to the U.S. Food and Drug Administration's Bacteriological Analytical Manual (FDA BAM) reference method. Results demonstrated statistically equivalent or superior performance of the BAX® System, validating the suitability of the BAX® System PCR assays to accurately detect *Salmonella* from an environmental scrub sampler with a wide spectrum neutralizer.

Introduction

Foodborne pathogens such as *Salmonella* can attach to various food contact surfaces within a processing environment serving as a source of product contamination (1). The control of *Salmonella* in these environments can be challenging, as they have demonstrated the ability to persist for many years. A properly designed environmental monitoring program (EMP) is therefore crucial for food manufacturers to find and eliminate this potentially resident pathogen. Routine sanitization and microbiological sampling are an integral part of this program and there are many sample collection devices and transport broths available (2). Choosing one that will neutralize a wide range of biocides and disinfectants used during sanitization without affecting the intended target growth and subsequent downstream detection methods is an important element in a successful EMP.

Sample Preparation and Enrichment

An overnight culture of *Salmonella* Typhimurium was serially diluted and inoculated onto unpaired 4" x 4" stainless steel surfaces to compare the BAX® System method and the FDA BAM reference method. Within each sample set, there were 20 low-level samples, 5 high-level samples and 5 uninoculated controls. *Enterococcus faecalis* was also inoculated on each test area but at 10X the concentration of *Salmonella* to represent competing flora. Surfaces were then dried for up to 24 hours.

For the BAX® System method, surfaces were swabbed with a 3M™ Environmental Scrub Sampler hydrated with 10 mL of Wide Spectrum Neutralizer and held at room temperature for 2 hours. Sponges were then homogenized with 90 mL of pre-warmed (35°C) Buffered Peptone Water (BPW) and incubated at 35°C for 18-24 hours.

For the reference method, surfaces were swabbed with a sponge hydrated with 10 mL of D/E Neutralizing broth and held at room temperature for 2 hours. Sponge were then enriched according to the procedures described in the FDA BAM Chapter 5 for *Salmonella*. See Figure 1.

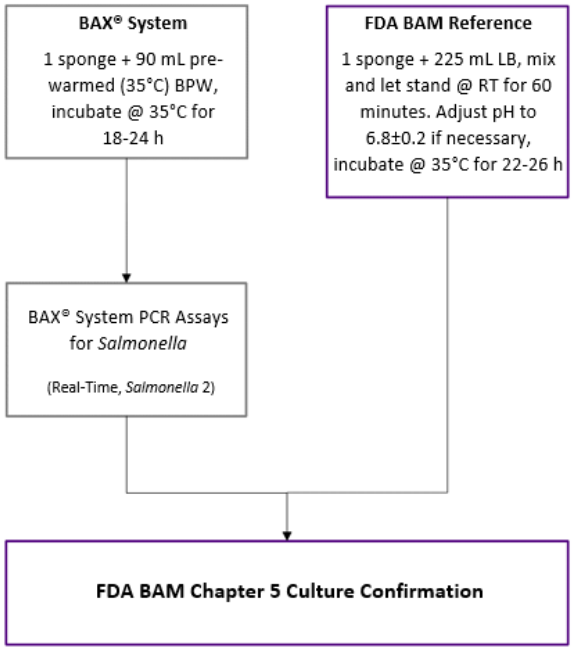

Figure 1. Unpaired study to compare the BAX® System method to the FDA BAM reference method for environmental samples.

Method

BAX® System Method

All samples were processed following the procedures described in the BAX® System Q7 User Guide for Real-Time *Salmonella* (KIT2006) and *Salmonella* 2 (KIT2011).

Reference Method

All samples were culture confirmed regardless of BAX® System results following the FDA BAM Chapter 5 for *Salmonella*.

Results

For BPW enrichments, both BAX® System Real-Time *Salmonella* and *Salmonella* 2 PCR assays returned presumptive positive results for 16/20 low spiked samples and 5/5 high spiked samples at 18 and 24 hours.

All of these results were determined to be correct by culture with no false positives or false negatives. The corresponding samples enriched using the reference method returned culture positive results for 11/20 low spiked samples and 2/5 high spiked samples.

Using the probability of detection (POD) to compare the BAX® System and the reference method, there were no significant differences observed for the low inoculation level since the 95% confidence interval contains zero. For the high inoculation level, the BAX® System method returned significantly more positives as the 95% confidence interval did not contain zero (Table 1).

Table 1. BAX® System Results vs. Reference Method Results

Matrix	*Salmonella* CFU/Test Area	N	Presumptive BAX® Results	Confirmed BAX® Results	Reference Method	$dPOD_C$ (BAX® vs Ref)	95% CI
Stainless (4" x 4")	0	5	0	0	0	0.00	-0.45, 0.45
	7.4	20	16	16	11	0.25	-0.04, 0.49
	74	5	5	5	2	0.60	0.03, 0.88

N = Number of test portions

X = Number of positive test portions

$dPOD_C$ = Difference between the BAX® System method confirmed and Reference POD values

95% CI = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

Conclusions

The results of this study indicate the suitability of the BAX® System Real-Time *Salmonella* PCR assay and the *Salmonella* 2 PCR assay to be used with the 3M™ Environmental Scrub Sampler with a Wide Spectrum Neutralizer. In both cases, the kits were able to accurately detect *Salmonella* species from stainless steel surfaces equivalent or superior to the reference method using the following enrichment protocol:

- Homogenize 1 sponge with 90 mL of pre-warmed (35°C) BPW and incubate at 35°C for 18-24 hours.

References

1. Speranza, B., Corbo, M. R., and Sinigaglia, M. 2011. Effects of Nutritional and Environmental Conditions on *Salmonella* sp. Biofilm Formation. Journal of Food Science. 76(1):M12-M16.
2. Li, F., Xian, Z., Kwon, H. J., Yoo, J., Burall, L., Chirtel, S. J., Hammack, T. S., and Chen, Y. 2020. Comparison of three neutralizing broths for environmental sampling of low levels of *Listeria monocytogenes* desiccated on stainless steel surfaces and exposed to quaternary ammonium compounds. BMC Microbiology. 20:333